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Cellulase activity and its relationship with host selection in the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae)

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Abstract: To investigate the relationship between cellulase activity and host selection, *Anoplophora glabripennis* (Motsch.) larvae from four different host trees (*Populus alba* var. *pyramidalis* Bunge, *Populus nigra* var. *thevestina* (Dode) Bean, *Populus simonii* × *Populus pyramidalis* cv. *opera* Hsu and *Salix matsudana* f. *lobato-glandulosa* Fang *et* Liu) and adults fed on five different tree species were selected. The enzyme activities of endoglucanase and β-glucosidase in the intestines of the insects were measured. The results showed that in the larvae, the activity of endoglucanase ranged from 1.36 to 2.71 μmol glucose/(g FW · h), and the activity of β-glucosidase ranged from 2.57 to 4.86 μmol glucose/(g FW · h). In the adults fed on different tree species, the activity of endoglucanase ranged from 4.08 to 9.27 μmol glucose/(g FW · h), and the activity of β-glucosidase ranged from 2.87 to 6.08 μmol glucose/(g FW · h). There are no significant differences in cellulase activities among the larvae from different host trees. However, the tree species which the adults fed upon has great effect on the activity of cellulase in the adults.

Key words: *Anoplophora glabripennis*; digestive enzyme; cellulase; enzyme activity; host selection

1 INTRODUCTION

The Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae: Lamiinae: Lamiini) is considered one of the most important forest pests in China. It has been reported from 25 provinces in China, extending from 21 to 43°N latitude and 100 to 127°E longitude (Yan, 1985). This species became a major pest in China after the susceptible *Populus* L. species (Salicaceae) were planted extensively throughout much of China as city trees, windbreaks, planting to supply firewood, and plantations for pulp (Hajek and Kalb, 2007). This beetle has established low density populations in Chicago, Illinois, New York City and Long Island, New York and several nearby areas of New Jersey in USA, and Toronto and Ontario in Canada. *A. glabripennis* has also been reported in Austria, Germany and France (Hajek, 2007).

In China, poplars (*Populus* spp.), willows (*Salix* spp.), maples (*Acer* spp.) and elm (*Ulmus* spp.) are the primary host tree species (Luo and Li, 1999). Larvae have been reported from at least 18 species of trees in 12 genera in North America (Morewood *et al.*, 2005). Larvae of ALB are wood borers, feeding in the phloem-cambium region during the first few instars and boring into the xylem in later instars. Adults are not sexually mature when they emerge from trees, and must undertake premature feeding for 9 - 15 d (Keena, 2002; Smith *et al.*, 2002), eating predominantly the bark of twigs (Hajek and Kalb, 2007). Beetles obtain nutrition by digesting the cellulose in their food. Cellulase is one of the most important digestive enzymes in their intestines, which digest cellulose (Suo *et al.*, 2004). Chen *et al.* (2002) have discovered that the ALB larvae have integrated cellulase complexes in their intestines, including endoglucanases, exoglucanase and β-glucosidase. Cellulase genes in the mulberry longicorn beetle *Apriona germari* and the yellow-spotted longicorn beetlep sacothea hilaris have already been cloned (Masahiro *et al.*, 2003; Wei *et al.*, 2006).

Our previous studies have concluded that there was no statistically significant difference in cellulase

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activities among ALB adults emerging from different host trees (Li *et al.*, 2008). In this study, cellulase activities of larvae from different host plants and adults fed on different tree species were assayed, in order to illustrate the relationship between cellulase activity and host selection of ALB. The results of this study will offer the scientific foundation for new ecological control methods, such as forestry measures and cellulase inhibitor for the prevention of further damages.

2 MATERIALS AND METHODS

2.1 Test beetles

Four different infected host tree species (*Populus alba* var. *pyramidalis* Bunge, *Populus nigra* var. *thevestina* (Dode) Bean, *Populus simonii* × *Populus pyramidalis* cv. *opera* Hsu and *Salix matsudana* f. *lobato-glandulosa* Fang *et* Liu) were cut down in Ningxia of China in October 2007. Mature larvae from four different host trees were collected in xylem, which were in similar size and instars.

Infected host trees of *Populus simonii* × *Populus pyramidalis* cv. *opera* Hsu were cut down and covered with wire netting in June 2008. Adults were caught when they emerged and came out of the emergence hole, and then put into a cage (wire netting sized 30 cm × 40 cm, one cage with one adult). We fed them with fresh branches of the trees of heaven (*Ailanthus altissima* Swingle), Chinese white poplar (*Populus tomentosa* Carr.), poplar opera (*Populus simonii* × *Populus pyramidalis* cv. *opera* Hsu), hankow willow (*Salix matsudana* Koidz.) and ash-leaf maple (*Acer negundo* Linn.) for three days. Adults were starved for three days as control.

2.2 Preparation of enzyme extracts

Larvae (ten larvae from each tree host species) were weighed and saved under −70℃ 24 h after extraction from the infested trees without giving different diets. Adults (five adults from each host tree species) were weighed and frozen after feeding for three days. After thawing at 4℃ for 4 h, they were dissected on ice. Intestines were taken out and put in a sterile mortar contained 2 mL ice-cold sodium acetate buffer (pH 5.2, 0.1 mol/L) and 0.5 g quartz sand immediately, homogenized and centrifuged at 12 000 r/min for 20 min at 4℃. The supernatants were collected and stored at −30℃ until the enzymes were assayed (Yin *et al.*, 1996; Chen *et al.*, 2002).

2.3 Determination of enzyme activities

The 1% (w/v) carboxymethylcellulose (CMC) and 1% (w/v) salicin were used as substrates in measurement of the activities of endoglucanase and β-glucosidase, respectively. Substrates were prepared and dissolved in sodium acetate buffer (pH 5.2, 0.1 mol/L).

Enzyme extracts were diluted 5-fold with the buffer. 100 μL diluted enzyme extract was incubated with 200 μL 1% substrate at 40℃ for 1 h. The reactions were stopped by adding 300 μL DNS reagents. After being mixed, the mixture were put into boiling water for 5 min, and then cooled at room temperature, and diluted 5-fold with distilled water. The reducing-sugar concentration was estimated by a spectrophotometer at 540 nm, with glucose as the standard for reducing-sugars. All samples were analyzed in triplicate and mean values were calculated. One unit of enzyme activity is defined as 1 μmol of reducing sugar (glucose equivalents) produced in 1 h by 1 g of beetle weight under this experimental conditions, *i. e.*, μmol glucose/(g FW · h) (Ghose, 1987; Yang *et al.*, 2003).

The reagents used in this study were: carboxymethylcellulose (CMC) from Sigma, subpackaged by Beijing Dingguo Biotechnology Ltd Company, salicin produced by Alfa Aesar in the USA. Other reagents used were analytical reagent.

2.4 Data statistics and analysis

The data for cellulase activity were statistically analyzed by a one-way analysis of variance (ANOVA) using the least significant difference test (LSD) post hoc pairwise multiple comparison procedure for contrast of the differences among treatment means. Results with $P < 0.05$ were considered significant with all statistical significance. All statistics were performed using SPSS 13.0.

3 RESULTS

There are no statistically significant differences in the enzyme activities of endoglucanase and β-glucosidase between mature larvae from different host species (Table 1). The order of enzyme activity is β-glucosidase > endoglucanase.

The activities of cellulase in ALB adults fed on different trees are showed in Figs. 1 and 2. Significant differences were found in the activities of endoglucanase between males and females fed on ash-leaf maple and control, respectively, and low activity of endoglucanase occured in both males and females fed on tree of heaven. Significant differences were found in β-glucosidase activity between males and females fed on ash-leaf maple and poplar opera, respectively, but there were no significant differences on other trees.

Table 1 Cellulase activities of ALB mature larvae from different host species

Host tree species	Enzyme activity [μmol glucose/(g FW · h)]	
	Endoglucanase	β-glucosidase
Populus alba var. *pyramidalis*	1.36 ± 0.45 a	2.57 ± 1.37 a
Populus nigra var. *thevestina*	2.71 ± 1.27 a	4.86 ± 2.53 a
Populus simonii × *Populus pyramidalis* cv. *opera*	2.35 ± 1.86 a	4.30 ± 3.65 a
Salix matsudana f. *lobato-glandulosa*	1.59 ± 0.93 a	3.99 ± 3.12 a

Data in table are mean values of ten replications ± standard deviations, and those in the same column followed by the same letters are not significantly different (ANOVA test, $P < 0.05$).

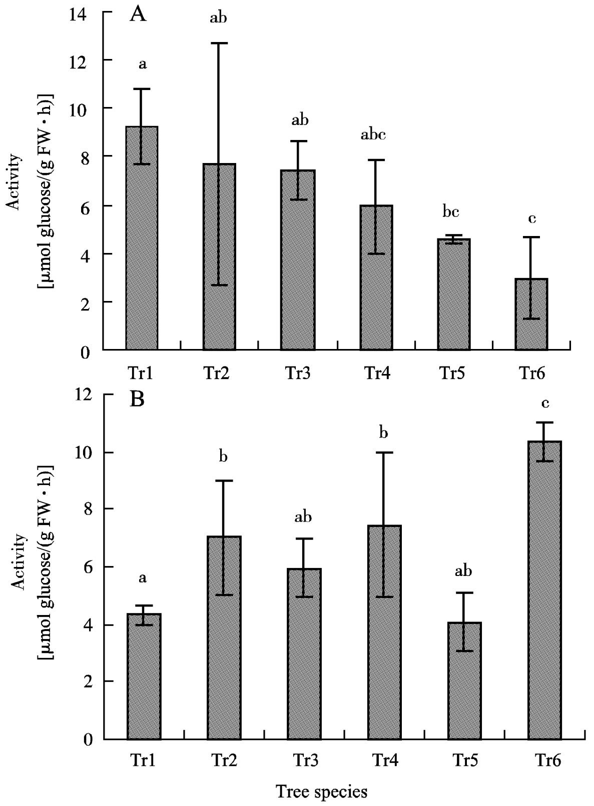

Fig. 1 Endoglucanase activities in ALB adults fed on different trees

A: Female; B: Male; Tr1: Ash-leaf maple; Tr2: Hankow willow; Tr3: Poplar opera; Tr4: Chinese white poplar; Tr5: Tree of heaven; Tr6: Control. The data were compared by ANOVA test. Bars labeled by different letters are significantly different ($P < 0.05$). The same for Fig. 2.

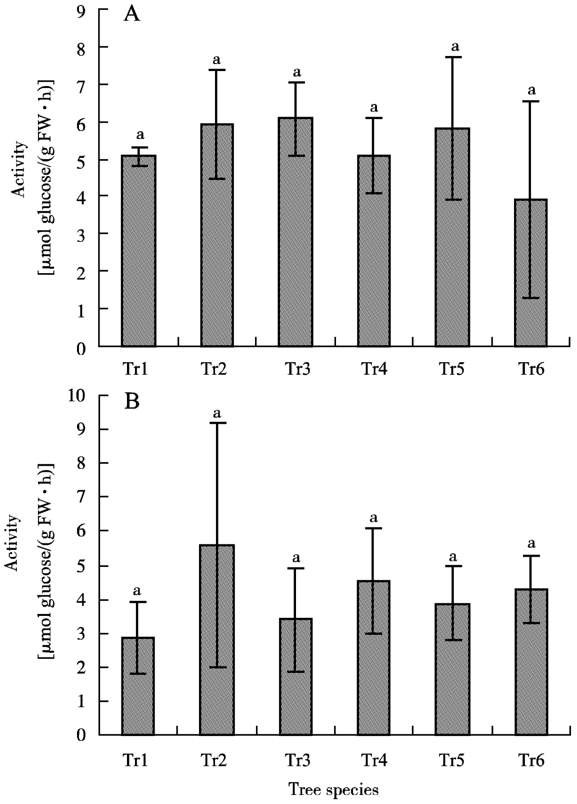

Fig. 2 β-glucosidase activities in ALB adults fed on different *populus* trees

4 DISCUSSION

It requires the combined reactions of endoglucanase, exoglucanase and β-glucosidase to decompose all of the natural cellulose, because none of the three kinds of cellulase can decompose natural cellulose effectively alone (Yan and Gao, 1995, 2000; Yang *et al.*, 2006). It is impossible to eliminate the function and influence of other cellulase completely in a reaction system, while measuring the enzyme activity of one kind of cellulase with particular substrate, the results in this paper only reflect the enzyme activity of the one which plays the key role.

There are no significant differences in the cellulase activities among larvae from different host species, based on the previous research results that there are no significant differences in the cellulase activities among adults emerging from different host species, it can be concluded that the content of cellulose in different host species and the cellulase activity in the beetle are not the main factors that restrict the distribution of ALB. It is a worthwhile issue for further research that the influence of nutritional components, secondary compounds, volatile compounds and anatomy structure of host plants on the effect of host selection in ALB adults. *Ceratitis capitata* larvae prefer to select the host which has greater quantities of solute and sugars, so greater nutritive value (Pedro and Fernando, 1993). Maybe that is the reason why almost 40% of the observations found both sexes of ALB on sugar maple (Morewood *et al.*, 2004). Secondary compounds play a decisive role in the evolution of host associations of phytophagous insects (Susanne, 2001). As for ALB, the contents of tannin and phenol constituents are positively related to the resistance of tree (Li *et al.*, 2003). Among the four host tree species with different resistant abilities to *Anoplophora glabripennis*, the resistant host trees of *A. glabripennis* released more alcohols and aldehydes, while the susceptible host trees released more esters and terpenoids (Li *et al.*, 2002). The number of vessels and rays' width of tree are propitious, and the contents of cellulose, lignin, ash, radius of lignified cells and wall thickness of sclereids are negative to the harm of ALB (Li *et al.*, 2004).

This study found that, the kind of plants the adults fed on has great effect on the activity of cellulase as a kind of induced enzymes in adults, especially for endoglucanase. Host tree also can impact cellulase activity in ALB larvae. Larvae that fed on wood from a resistant host (*Pyrus calleryana*) showed suppressed total gut cellulase activity (Geib *et al.*, 2009). Low activity of endoglucanase was found in both male and female fed on tree of heaven. However, it is hard to conclude that the low activity is caused by starvation or some inhibitory effects from the tree of heaven. The most feeding trace appeared on the ash-leaf maple, while there was little even no feeding trace on the tree of heaven. Compared to other tree species, ash-leaf maple has higher sugar content. In China, maples are usually planted as trap trees to protect more-valuable hardwoods (Luo *et al.*, 2003). The most preferred by ALB in North America are maple species (Haack *et al.*, 1997).

The secondary metabolites present in plants, providing protection against predators, pathogens and invaders because of their anti-microbial activity. The majority of these compounds fall into the category of lignins, tannins, saponins, volatile essential oils, alkaloids, *etc.* (Kamra *et al.*, 2006). α-pinene, β-pinene and α-terpinene from tree of heaven had a strong toxic effect on ALB adults (Cao and Zhao, 1997). The efficacy for two cellobiose-based cellulase inhibitors, cellobioimidazole (CBI) and fluoromethylcellobiose (FMCB), has been approved in termite (Zhou *et al.*, 2008). Secondary compounds from tree of heaven are potential inhibitor as novel ALB control agents.

As the structure of natural cellulose and the substrates in measurement are not identical, in addition, the digestion process was assisted by chewing of mouthparts and peristalsis of intestine under natural condition, the activity of cellulase detected in the laboratory can not reflect the digestive ability of ALB to natural cellulose completely. In view of this, the most significance of this study will lie in reflecting the variation trend of cellulase activity in ALB and provide the scientific evidence for controlling by utilizing cellulase characteristics.

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光肩星天牛体内纤维素酶活性及其与寄主选择的关系

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摘要：为了揭示光肩星天牛 *Anoplophora glabripennis* Motschulsky 纤维素酶与寄主选择的关系，以 4 种不同寄主树种（新疆杨 *Populus alba* var. *pyramidalis* Bunge、箭杆杨 *Populus nigra* var. *thevestina* (Dode) Bean、合作杨 *Populus simonii* × *Populus pyramidalis* cv. *opera* Hsu 和漳河柳 *Salix matsudana* f. *lobato-glandulosa* Faug *et* Liu 来源的光肩星天牛幼虫，和以取食 5 种不同树种（臭椿 *Ailanthus altissima*、毛白杨 *Populus tomentosa*、合作杨、旱柳 *Salix matsudana* Koidz. 和复叶槭 *Acer negundo* Linn.）的光肩星天牛成虫为实验对象，测定其纤维素酶活性。结果表明：不同寄主来源的光肩星天牛幼虫内切-β-1,4-葡聚糖酶活性在 1.36 ~ 2.71 μmol 葡萄糖/(g FW · h)之间，β-葡萄糖苷酶活性在 2.57 ~ 4.86 μmol 葡萄糖/(g FW · h)之间；取食不同树种的光肩星天牛成虫内切-β-1,4-葡聚糖酶活性在 4.08 ~ 9.27 μmol 葡萄糖/(g FW · h)之间，β-葡萄糖苷酶活性在 2.87 ~ 6.08 μmol 葡萄糖/(g FW · h)之间。不同寄主树种来源的光肩星天牛幼虫体内纤维素酶活性无显著性差异，取食与否以及取食树种的不同对光肩星天牛成虫纤维素酶活有较大影响。

关键词：光肩星天牛；消化酶；纤维素酶；酶活性；寄主选择

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